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 ********** STN Columbus **********
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L1         1 S LDAO
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L2      1579 S L1
L3         1 S HEPATITIS AND L2
L4      9599 S REFOLD? OR RENATUR?
L5         3 S L2 AND L4
L6       115 S PROTEIN AND L2
L7         8 S INCLUSION AND L2
   FILE 'REGISTRY' ENTERED AT 14:30:58 ON 04 MAR 2003
=> d l3 bib abs

L3  ANSWER 1 OF 1  CA  COPYRIGHT 2003 ACS
AN  137:29823  CA
TI  Purification of active NS2/3 protease of  ***hepatitis***  C virus from
    inclusion bodies
IN  Thibeault, Diane; Lamarre, Daniel; Maurice, Roger; Pilote, Louise; Pause,
    Arnim
PA  Boehringer Ingelheim (Canada) Ltd., Can.
SO  PCT Int. Appl., 67 pp.
    CODEN: PIXXD2
DT  Patent
LA  English
FAN.CNT 1
    PATENT NO.      KIND  DATE        APPLICATION NO.  DATE
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PI  WO 2002048375   A2    20020620    WO 2001-CA1796   20011213
      W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
         CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
         GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
         LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL,
         PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG,
         US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
      RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,
         CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,
         BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
    AU 2002024688   A5    20020624    AU 2002-24688    20011213
    US 2002192640   A1    20021219    US 2001-17736    20011214
PRAI US 2000-256031P  P   20001215
    WO 2001-CA1796    W   20011213
AB  A method for producing a refolded, inactive form of recombinantly produced
    NS2/3 protease by purifying the protease from inclusion bodies in the
    presence of a chaotropic agent and refolding the purified protease by
    contacting it with a reducing agent and lauryldiethylamine oxide (LDAO) in
    the presence of reduced concn. of chaotropic agent or polar additive. The
    invention further comprises a method for activating this refolded inactive
    NS2/3 protease by adding an activation detergent. This method produces
    large amts. of the active NS2/3 protease to allow small mols. and ligands
    to be screened as potential inhibitors of NS2/3 protease, which may be
    useful as therapeutic agents against HCV. Protocols for the manuf. and
    resolubilization of the enzyme as inclusion bodies in Escherichia coli are
    described in detail.

=> d l7 6 bib abs

L7  ANSWER 6 OF 8  CA  COPYRIGHT 2003 ACS
AN  111:152144  CA
TI  Recovery of proteins from cells using surfactants
IN  Patroni, Joseph John; Brandon, Malcolm Roy
PA  Bunge (Australia) Pty. Ltd., Australia
SO  Eur. Pat. Appl., 5 pp.
    CODEN: EPXXDW
DT  Patent
LA  English
FAN.CNT 1
    PATENT NO.      KIND  DATE        APPLICATION NO.  DATE
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PI  EP 295859       A2    19881221    EP 1988-305404   19880614
    EP 295859       A3    19900418
    EP 295859       B1    19941117
      R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE
    AU 8817632      A1    19881215    AU 1988-17632    19880610
    AU 609824       B2    19910509
    ZA 8804212      A     19890329    ZA 1988-4212     19880613
    US 4992531      A     19910212    US 1988-206006   19880613
    DK 8803221      A     19881217    DK 1988-3221     19880614
    CA 1305284      A1    19920714    CA 1988-569448   19880614
    ES 2065911      T3    19950301    ES 1988-305404   19880614
    NO 8802635      A     19881219    NO 1988-2635     19880615
    CN 88103725     A     19881228    CN 1988-103725   19880615
    CN 1032425      B     19960731
    JP 01047389     A2    19890221    JP 1988-147909   19880615
    KR 126767       B1    19971229    KR 1988-7253     19880616
PRAI AU 1987-2472   A     19870615
AB  Proteins in host cells, e.g. those in  ***inclusion***  bodies, are
    recovered using cationic, anionic, or zwitterionic surfactants.
    Escherichia coli contg. methionine-porcine growth hormone in
     ***inclusion***  bodies were treated with 20 wt./vol.%
    cetyltrimethylammonium bromide for 1 h at room temp. Upon centrifugation,
    a pellet was obtained which contained no  ***inclusion***  bodies (as
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detd. by electron microscopy).

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L1 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2003 ACS
RN 1643-20-5 REGISTRY
CN 1-Dodecanamine, N,N-dimethyl-, N-oxide (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Dodecylamine, N,N-dimethyl-, N-oxide (6CI, 8CI)
OTHER NAMES:
CN 101CG
CN Admox 12
CN Ammonyx AO
CN Ammonyx C10 Amine Oxide
CN Ammonyx DMCD 40
CN Ammonyx LO
CN Amphitol 20N
CN Aromox DM 12D
CN Aromox DM 12D-W
CN Aromox DM 12W
CN Aromox DMCD
CN Atlas CD 413
CN Conco XAL
CN Cyclomox L
CN DDNO
CN Dimethylaurylamine oxide
CN Dimethyldodecylamine oxide
CN Dimethyllaurylamine oxide
CN Dodecyldimethylamine oxide
CN Emal 20N
CN Emcol L
CN Emcol LO
CN Empigen OB
CN Incromine oxide L
CN Lauramine oxide
CN Laurylamine oxide
CN Lauryldimethylamine N-oxide
CN Lauryldimethylamine oxide
CN ***LDAO***
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CN N,N-Dimethyldodecylamine oxide
CN N-Dodecyl-N,N-dimethylamine oxide
CN n-Dodecyldimethylamine oxide
CN N-Lauryl-N,N-dimethylamine oxide
CN N-Lauryldimethylamine N-oxide
CN Ninox DMCD 40
CN Ninox L
CN Nissan Unisafe A-LM
CN Oxamin LO
CN Oxidet DM 20
CN Rewominox L 408
CN Rhodamox L
CN Rhodamox LO
CN Schercamox DML
CN Softamine L
CN Tomah AO 728
CN Unisafe A-LM
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DR 177162-47-9, 163221-07-6, 135526-66-8, 73502-08-6, 160714-02-3,
311814-25-2
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CI COM
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MEDLINE,
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USPAT2,
USPATFULL
(*File contains numerically searchable property data)
Other Sources: DSL**, EINECS**, TSCA**
(**Enter CHEMLIST File for up-to-date regulatory information)

/ Structure 1 in file .gra /
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12 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
1574 REFERENCES IN FILE CAPLUS (1962 TO DATE)
21 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

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COPYRIGHT (C) 2003 American Chemical Society (ACS)
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PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
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STRUCTURE FILE UPDATES: 3 MAR 2003 HIGHEST RN 496834-05-0
DICTIONARY FILE UPDATES: 3 MAR 2003 HIGHEST RN 496834-05-0
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E1 THROUGH E1 ASSIGNED
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On June 9, 2002, MEDLINE was reloaded. See HELP RLOAD for details.
MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
MeSH 2003 vocabulary. See http://www.nlm.nih.gov/mesh/summ2003.html
for a description on changes.
This file contains CAS Registry Numbers for easy and accurate
substance identification.
=> S L8
L9 59 L8
=> s purif? and l9
609884 PURIF?
L10 20 PURIF? AND L9

=> d L10 b abs

L10 ANSWER 2 OF 20 MEDLINE
AN 2002209524 MEDLINE
DN 21940604 PubMed ID: 11943219
TI Self-assembly of ATP synthase subunit c rings.
AU Arechaga Ignacio; Butler P Jonathan G; Walker John E
CS The Medical Research Council Dunn Human Nutrition Unit, Hills Road, CB2 2YK, Cambridge, UK.
SO FEBS LETTERS, (2002 Mar 27) 515 (1-3) 189-93.
Journal code: 0155157. ISSN: 0014-5793.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200205
ED Entered STN: 20020412
Last Updated on STN: 20020511
Entered Medline: 20020510
AB Subunit c of the H(+) transporting ATP synthase is an essential part of its membrane domain that participates in transmembrane proton conduction. The annular architecture of the subunit c from different species has been previously reported. However, little is known about the type of interactions that affect the formation of c-rings in the ATPase complex. Here we report that subunit c over-expressed in Escherichia coli and

purified in non-ionic detergent solutions self-assembles into annular structures in the absence of other subunits of the complex. The results suggest that the ability of subunit c to form rings is determined by its primary structure.

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CA SUBSCRIBER PRICE 0.00 -1.24
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b 155
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File 155:MEDLINE(R) 1966-2003/Feb W4
(c) format only 2003 The Dialog Corp.

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S2 0 NS2/3(W)PROTEASE
S3 15 NS2(2W)PROTEASE
S4 50 NS2(W)3
S5 73251 PROTEASE OR PROTEINASE
S6 25 S4 AND S5
? t s6/7/3 5 6 13 18-20
6/7/3
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
12710074 21588263 PMID: 11591719
In vitro characterization of a purified NS2/3 protease variant of hepatitis C virus.
Thibeault D; Maurice R; Pilote L; Lamarre D; Pause A
Department of Biological Sciences, Boehringer Ingelheim (Canada) Ltd., Research and Development, Laval, Quebec H7S 2G5, Canada. dthibeault@lav.boehringer-ingelheim.com
Journal of biological chemistry (United States) Dec 7 2001, 276 (49) p46678-84, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The cleavage of the hepatitis C virus polyprotein between the nonstructural proteins NS2 and NS3 is mediated by the NS2/3 protease, whereas the NS3 protease is responsible for the cleavage of the downstream proteins. Purification and in vitro characterization of the NS2/3 protease has been hampered by its hydrophobic nature. NS2/3 protease activity could only be detected in cells or in in vitro translation assays with the addition of microsomal membranes or detergent. To facilitate purification of this poorly characterized protease, we truncated the N-terminal hydrophobic domain, resulting in an active enzyme with improved biophysical properties. We define a minimal catalytic region of NS2/3 protease

retaining autocleavage activity that spans residues 904-1206 and includes the C-terminal half of NS2 and the N-terminal NS3 protease domain. The NS2/3 (904-1206) variant was purified from Escherichia coli inclusion bodies and refolded by gel filtration chromatography. The purified inactive form of NS2/3 (904-1206) was activated by the addition of glycerol and detergent to induce autocleavage at the predicted site between Leu(1026) and Ala(1027). NS2/3 (904-1206) activity was dependent on zinc ions and could be inhibited by NS4A peptides, peptides that span the cleavage site, or an N-terminal peptidic cleavage product. This NS2/3 variant will facilitate the development of an assay suitable for identifying inhibitors of HCV replication.

Record Date Created: 20011203

6/7/5

DIALOG(R)File 155:MEDLINE(R)

11362000 21443988 PMID: 11559826

Characterization of the hepatitis C virus NS2/3 processing reaction by using a purified precursor protein.

Pallaoro M; Lahm A; Biasiol G; Brunetti M; Nardella C; Orsatti L; Bonelli F; Orru S; Narjes F; Steinkuhler C

Department of Biochemistry, Istituto di Ricerche di Biologia Molecolare "P. Angeletti," Pomezia, Italy.

Journal of virology (United States) Oct 2001, 75 (20) p9939-46, ISSN 0022-538X Journal Code: 0113724

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The NS2-NS3 region of the hepatitis C virus polyprotein encodes a proteolytic activity that is required for processing of the NS2/3 junction. Membrane association of NS2 and the autocatalytic nature of the NS2/3 processing event have so far constituted hurdles to the detailed investigation of this reaction. We now report the first biochemical characterization of the self-processing activity of a purified NS2/3 precursor. Using multiple sequence alignments, we were able to define a minimal domain, devoid of membrane-anchoring sequences, which was still capable of performing the processing reaction. This truncated protein was efficiently expressed and processed in Escherichia coli. The processing reaction could be significantly suppressed by growth in minimal medium in the absence of added zinc ions, leading to the accumulation of an unprocessed precursor protein in inclusion bodies. This protein was purified to homogeneity, refolded, and shown to undergo processing at the authentic NS2/NS3 cleavage site with rates comparable to those observed using an in vitro-translated full-length NS2/3 precursor. Size-exclusion chromatography and a dependence of the processing rate on the concentration of truncated NS2/3 suggested a functional multimerization of the precursor protein. However, we were unable to observe trans cleavage activity between cleavage-site mutants and active-site mutants. Furthermore, the cleavage reaction of the wild-type protein was not inhibited by addition of a mutant that was unable to undergo self-processing. Site-directed mutagenesis data and the independence of the processing rate from the nature of the added metal ion argue in favor of NS2/3 being a cysteine protease having Cys993 and His952 as a catalytic dyad. We conclude that a purified protein can efficiently reproduce processing at the NS2/3 site in the absence of additional cofactors.

Record Date Created: 20010917

6/7/6

DIALOG(R)File 155:MEDLINE(R)

11339775 21405473 PMID: 11513846

Reconstitution of hepatitis C virus protease activities in yeast.

Mak P; Palant O; Labonte P; Plotch S

Molecular Biology and Virology Section, Wyeth-Ayerst Research, 401 N. Middletown Road, Pearl River, NY 10965, USA. makp@war.wyeth.com

FEBS letters (Netherlands) Aug 10 2001, 503 (1) p13-8, ISSN 0014-5793 Journal Code: 0155157

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The hepatitis C virus (HCV) protease genes (NS2/3 and NS3) were expressed in yeast with their natural substrates fused to a ligand-dependent transcriptional activator, the retinoic acid receptor (RARbeta). RARbeta can activate transcription in yeast cells in response to retinoic acids. We hypothesized that cis-cleavage at the NS2-3 or NS3-4A junctions by the appropriate HCV proteases would release RARbeta, thereby activating transcription of a reporter gene. Our results from Western blot analyses and reporter gene activation indicate that the wild-type NS2/3 and NS3 enzymes are catalytically active in yeast cells, whereas mutations in the catalytic domain of NS2(C993V) and NS3(S1165A) lead to inactive enzymes. We conclude that HCV NS2/3 and NS3 protease activities can be reconstituted in yeast.

Record Date Created: 20010821

6/7/13

DIALOG(R)File 155:MEDLINE(R)

10142656 99127866 PMID: 9930676

Engineering, characterization and phage display of hepatitis C virus NS3 protease and NS4A cofactor peptide as a single-chain protein.

Dimasi N; Pasquo A; Martin F; Di Marco S; Steinkuhler C; Cortese R; Sollazzo M

Istituto di Ricerche di Biologia Molecolare, Pomezia (Rome), Italy.
Protein engineering (ENGLAND) Dec 1998, 11 (12) p1257-65, ISSN 0269-2139 Journal Code: 8801484
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The polyprotein encoded by hepatitis C virus (HCV) genomic RNA is processed into functional polypeptides by both host- and virus-encoded proteases. The HCV-encoded NS3 protease and its cofactor peptide NS4A form a non-covalent complex, which participates in processing the viral polyprotein. This proteolytic activity is believed to be essential for virus proliferation and thus the NS3 protease is a prime target for developing anti-HCV pharmacological agents. Recent X-ray crystallography structural studies have revealed the nature of this non-covalent complex between NS3 protease and the 'active' central segment of NS4A, providing the opportunity to design a single-chain polypeptide. To this end, the DNA sequence encoding for the NS4A peptide (residues 21-34) was genetically fused via a short linker, capable of making a beta-turn, to the N-terminus of the NS3 protease domain. This engineered single-chain NS3-protease (scNS3) is fully active with kinetic parameters virtually identical with those of the NS3/ NS4A non-covalent complex. Moreover, the scNS3 protease can be displayed on filamentous phage and affinity selected using an immobilized specific inhibitor. The scNS3 expressed as a soluble protein and in a phage-display format facilitates enzyme engineering for further structural studies and in vitro selection of potential drug-resistant mutants. These are important steps towards developing effective anti-protease compounds.
Record Date Created: 19990323

6/7/18
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
09698910 98111692 PMID: 9450039
Hepatitis C virus NS2-3 proteinase.
Wilkinson C S
Roche Discovery Welwyn, Welwyn Garden City, Herts, England.
Biochemical Society transactions (ENGLAND) Nov 1997, 25 (4) pS611, ISSN 0300-5127 Journal Code: 7506897
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Record Date Created: 19980413

6/7/19
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.
09504675 97404642 PMID: 9261354
In vitro study of the NS2-3 protease of hepatitis C virus.
Pieroni L; Santolini E; Fipaldini C; Pacini L; Migliaccio G; La Monica N
I.R.B.M. Instituto di Ricerche di Biologia Molecolare P. Angeletti, Italy.
Journal of virology (UNITED STATES) Sep 1997, 71 (9) p6373-80, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Processing at the C terminus of the NS2 protein of hepatitis C virus (HCV) is mediated by a virus-encoded protease which spans most of the NS2 protein and part of the NS3 polypeptide. In vitro cotranslational cleavage at the 2-3 junction is stimulated by the presence of microsomal membranes and ultimately results in the membrane insertion of the NS2 polypeptide. To characterize the biochemical properties of this viral protease, we have established an in vitro assay whereby the NS2-3 protease of HCV BK can be activated posttranslationally by the addition of detergents. The cleavage proficiency of several deletion and single point mutants was the same as that observed with microsomal membranes, indicating that the overall sequence requirements for proper cleavage at this site are maintained even under these artificial conditions. The processing efficiency of the NS2-3 protease varied according to the type of detergent used and its concentration. Also, the incubation temperature affected the cleavage at the 2-3 junction. The autoproteolytic activity of the NS2-3 protease could be inhibited by alkylating agents such as iodoacetamide and N-ethylmaleimide. Metal chelators such as EDTA and phenanthroline also inhibited the viral enzyme. The EDTA inhibition of NS2-3 cleavage could be reversed, at least in part, by the addition of ZnCl2 and CdCl2. Among the common protease inhibitors tested, tosyl phenylalanyl chloromethyl ketone and soybean trypsin inhibitor inactivated the NS2-3 protease. By means of gel filtration analysis, it was observed that the redox state of the reaction mixture greatly influenced the processing efficiency at the 2-3 site and that factors present in the rabbit reticulocyte lysate, wheat germ extract, and HeLa cell extract were required for efficient processing at this site. Thus, the in vitro assay should allow further characterization of the biochemical properties of the NS2-3 protease of HCV and the identification of host components that contribute to the efficient processing at the 2-3 junction.
Record Date Created: 19970917

6/7/20
DIALOG(R)File 155:MEDLINE(R)
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09281493 97201561 PMID: 9049347

Identification of the protease domain in NS3 of hepatitis C virus.
Han D S; Hahm B; Rho H M; Jang S K
Department of Life Science, Pohang University of Science and Technology, Kyungbuk, Korea.
Journal of general virology (ENGLAND) Apr 1995, 76 (Pt 4) p985-93, ISSN 0022-1317 Journal Code: 0077340
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
NS3 of hepatitis C virus (HCV) is a serine protease that carries out the proteolytic processing of the nonstructural proteins of the HCV polyprotein. Deletion analysis of the N terminus of NS2,3,4 fusion protein revealed that the N-terminal boundary of the active protease resides between amino acids 1050 and 1083. The processing patterns of internal deletion mutants of NS2,3,4 indicated that the C terminus of the enzymically active protease resides between amino acids 1115 and 1218. The N- and C-terminal boundaries of the protease were also confirmed by determining the trans-cleavage activity of internally deleted NS3,4. NS3 protease activity was inhibited by Cu2+ but was slightly enhanced by Zn2+. This report provides a possible approach for development of antiviral agents based on protease inhibitors.
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